PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicant | : Kitagawa, Harukazu et al. | Confirmation No. 7304 |
| Application No. | : 10/590,761 | |
| Filed | : August 24, 2006 | |
| Title | : AN IMMUNOSTIMULATORY OLIGONUCLEOTIDE THAT INDUCES INTERFERON ALPHA | |
| Grp./Div. | : 1633 | |
| Examiner | : Epps Smith, Janet L. | |
| Docket No. | : 58270/A400 | |

**DECLARATION OF HARUKAZU KITAGAWA UNDER 37 CFR 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Post Office Box 7068
Pasadena, CA 91109-7068

Commissioner:

I, Harukazu Kitagawa, a resident of Sakai-gun, Fukui, Japan, declare and state as follows:

1. I am a named inventor of the invention disclosed and claimed in the subject patent application, U.S. Patent Application Serial No. 10/590,761. I received a Doctor of Philosophy degree in Infection immunology from the Fukui Medical University in 1990. I have been employed by EMORI & CO., LTD since May, 2000. My responsibilities include research in oligonucleotides and Infection immunology, and I consider myself an expert in this area.

2. I am familiar with European Patent Application No. 468520 A2 (Tokunaga et al.) and International Patent Publication WO 2002/02172 A1 (Kaji et al.), which have been cited by the patent examiner of the United States Patent and Trademark Office against the subject patent application.

3. Tokunaga et al. describes 6- to 80-chain length sequences centering on the GACGTC base sequence as a palindrome structure. Among these, many 30-chain length sequences are described, but the guanine (G) addition is not investigated.

4. Kaji et al. investigate 4- to 14-chain length sequences centering on 6-chain length AACGTT as a palindrome sequence. Among those investigated, the sequences comprising the

palindrome sequence flanked by only four guanylic acid residues (G4) on both the 5' and 3' sides, or only 5' side are compared with AACGTT palindrome alone (Tables 1-4 of Kaji et al.).

5. The experimental methods of Tokunaga et al. and Kaji et al. are not different from the classical method of adding guanines (Gs) for the purpose of increasing and facilitating the uptake of an oligonucleotide into cells. Therefore, having an oligonucleotide with the unequal flanking of nine 5' guanines and one 3' guanine as found in the presently claimed SEQ ID NO: 19 is not suggested by Tokunaga et al. in view of Kaji et al. The experimental designs of both Tokunaga et al. and Kaji et al. do not take into consideration the number and, most importantly, the position of the Gs. Therefore, with reference to the level of academic recognition of an immunostimulatory oligonucleotide at the time Tokunaga et al. and Kaji et al. were published, a person skilled in the art could not have predicted that the activity thereof largely varies depending on the number and position of the Gs as well as the palindromic sequence, itself.

6. Two years after the the priority date of Tokunaga et al., the same group published that, "*The relationships between the sequence and the activity of the palindrome are not very clear at present.*" (page 1129, right column paragraph, line 25 of *Jpn J Cancer Res* 83(11): 1128-1131(1992), Kuramoto E., Yamamoto S., Tokunaga T.) Accordingly, the existence of the receptor, not to mention the operational mechanism, was unknown at the time that Tokunaga et al. was published in 1992. The present invention recognizes the importance of the receptor and the steric structure defined by the number and position of the cytophilic Gs added to the palindromic sequence.

7. **Exhibit A** (enclosed herewith) shows data from the claimed SEQ ID NO: 19 G9-GACGATCGTC-G1), G10-10 (G10- GACGATCGTC-G10) and #2006 (24-chain length oligonucleotide, tcgtcgttttgtcgttttgtcgtt, phosphorothioate linkage). Each of these oligonucleotides was administered to the abdominal cavity of eight week old C57BL/6 mice, and spleen tissue was collected at 0, 4, 10, and 17 days (D0, D4, D10, D17) after administration. RNA was extracted from the spleen tissue using the AGPC (acid-guanidium-phenol-chloroform) method, and the mRNA expression of TNF-$\alpha$, IFN-$\alpha_1$, IFN-$\gamma$, T-bet, IL-12 p35, and IL-10 was

investigated by Real Time PCR (Figures 1A-1F, respectively). The mice receiving G10-10 and #2006 showed that the expression of the various species of cytokines increased four days after administration, but decreased after ten days. In the mice receiving G9-1, the expression at ten days after administration was observed to be comparable with four days after administration. The superior results of SEQ ID NO: 19 as shown in th specification (e.g., Figure 12) and in the data shown here in Exhibit A, are evidence that the G9-1 pattern of guanines to this palindrome is a result-effective variable that was not recognized by the Tokunaga et al. and Kaji et al.

8. It is thought that this sustained cytokine induction effect advantageously operates in the induction of Th1 immunity. Thus, it is thought that the presently claimed SEQ ID NO: 19 (G9-1) provides an advantage in the development of the prevention and treatment of infectious diseases from microorganisms. Given that IFN-$\alpha_1$, having an anti-viral effect, is continuously expressed in G9-1 mice, it seems a synergistic effect between TNF-$\alpha$ and IFN-$\gamma$ is created.

9. Furthermore, inflammatory cytokines such as TNF-$\alpha$, IFN-$\gamma$, and IL-1$\beta$ play an important role in the protection against infection of tuberculosis. In mice receiving G9-1, the expression of these cytokines was amplified and sustained. Thus, it is suggested that G9-1 is effective in the prevention and therapy of tuberculosis infection. In view of the possible synergistic effect between TNF-$\alpha$ and INF-$\gamma$, it is thought that a therapy and a preventative effect for microbial infections other than tuberculosis can be anticipated for SEQ ID NO: 19.

10. It is important to note that T-bet induces the differentiation of Th0 cells to Th1 cells. Th1 cells activate microphages and neutrophils through the production of cytokines such as IFN-$\gamma$ and TNF-$\beta$, thus, inducing the expression of T-bet is thought to be related to strengthening the biological defense against tuberculosis. The data in Exhibit A show that in G9-1 administered mice, T-bet is continuously expressed, suggesting the possibility that G9-1 effectively operates in the enhancement of differentiation of Th1 cells. G9-1 also induces the expression of IL-10 which induces Th2 cells to differentiate. Therefore, it is possible that G9-1 has the additioanl effect of controlling the expression of T-bet. Further, G9-1 induces cytokine production such as

IL-4, IL-5, IL-6 (data not shown) and, thus, it is predicted to have an amplification effect of antibody-mediated immunity.

11. SEQ ID NO: 19 (G9-1) confers a strong and sustained induction of cytokines that is superior to oligonucleotides having the same palindromic sequence and flanking guanines that are not of the G9-1 pattern. The immunostimulatory effects of SEQ ID NO 19 are continuous, and are thought to be effective in the development of novel prevention and therapeutic medicaments for infectious diseases.

12. I declare that all statements made herein are of my own knowledge, are true, and that all statements made on information and belief are believed to be true. The statements herein are made with the knowledge that willful false statements are punishable by fine, imprisonment, or both, under Title 18, § 1001 of the United States Code, and that such willful statements may jeopardize the validity of this application or any patent issued on this application.

Date August 19, 2009. By Harukazu Kitagawa.

Harukazu Kitagawa

**EXHIBIT A USSN 10/590,761**

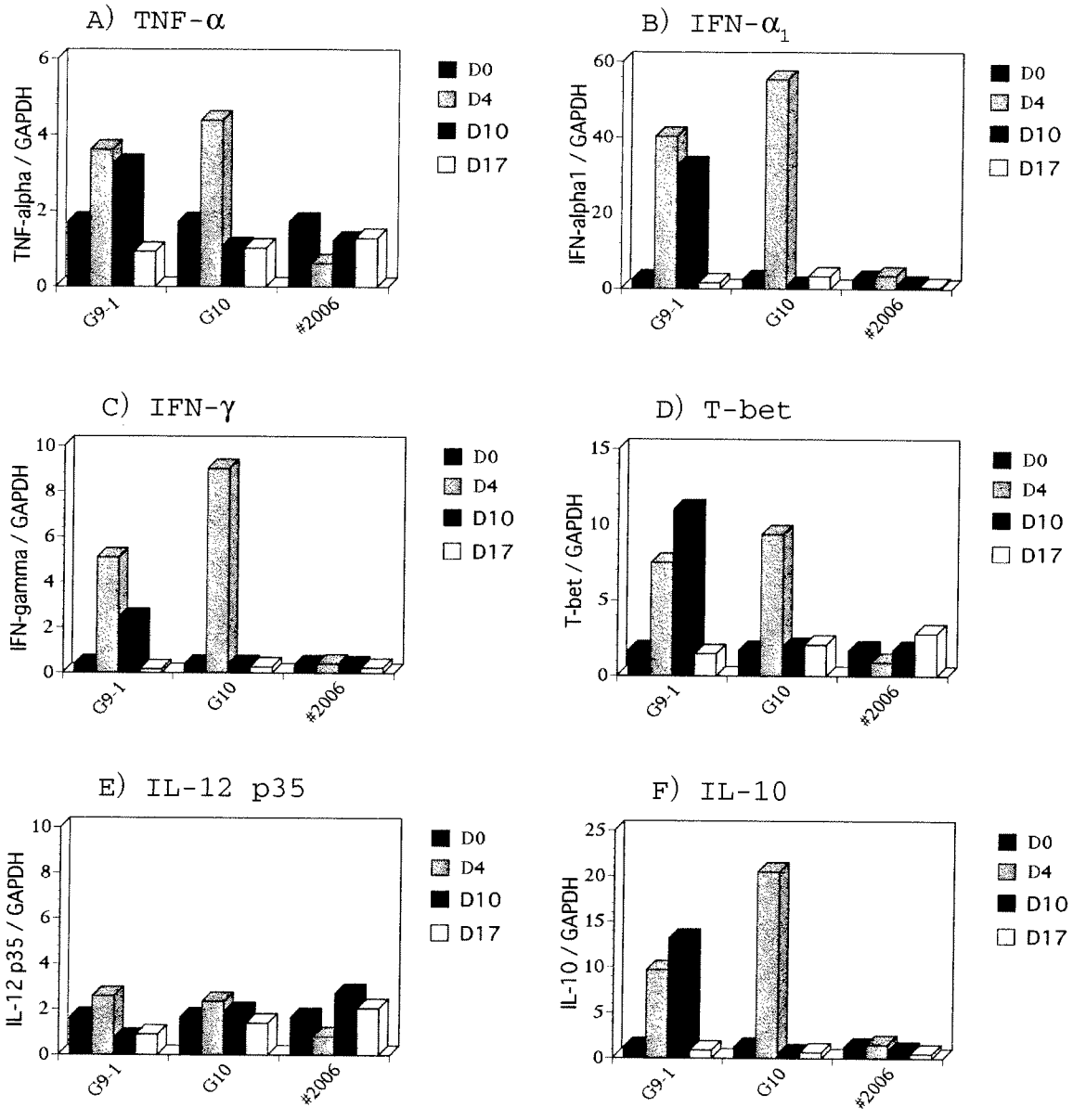


FIG. 1. Expression of each species of cytokine mRNA after oligionucleotide administration